REGULATORY MECHANISMS OF IMMUNE CELLS IN SPONGES. *Memoirs of the Queensland Museum 44: 248. 1999*:- Gray cells, large granular wandering cells present throughout the tissues of many species of sponges, have been identified as immunocytes in two species of sponges, *Microciona prolifera* and *Callyspongia diffusa*. When the tissues of two different sponge individuals are apposed, the gray cells accumulate at the boundary of contact at the time of tissue rejection. I have suggested that these cells may be viewed as the most primordial examples of evolutionary predecessors of the well-known vertebrate lymphocytes. This comparison implies that gray cells share features of vertebrate lymphocytes and I have examined this idea with studies on two prominent aspects of activation of T and B cells. The primary signalling event upon activation of a lymphocyte by recognition of an appropriate immune target is the synthesis and release of cytokines that alert and coordinate the activity of other lymphocytes in the surrounding tissue and throughout the body. In addition the activation of lymphocytes involves internal second messenger pathways converging on the transcription factor, NFkB, that are inhibited by Cyclosporin A, a drug often used medically to prevent rejection in human transplants. Using Boyden Chamber assays, the assays originally used to identify vertebrate immune system cytokines, I have succeeded in establishing in *M. prolifera* that contact with foreign tissue stimulates the release of cytokines activating the migration of gray cells toward the contacting tissue. Similarly, doses of Cyclosporin A commonly used to inhibit the activation of vertebrate T cells, suppresses histoincompatibility in *M. prolifera* and allows the healing together of tissue from two individual sponges that would normally undergo tissue rejection. These results provide further evidence that the foundations of the cellular immune system of animals were already established in the sponges and that study of gray cells will provide insight into the course of evolution of animal immunity. ❐ *Porifera, immunology, immunocytes, gray cells.*

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***NEGOMBATA MAGNIFICA* – A MAGNIFICENT (CHEMICAL) PET.** *Memoirs of the Queensland Museum 44: 248. 1999*:- *Negombata magnifica* (*Latrunculia*) is a Red Sea sponge known to produce the toxin latrunculin (Lat). Since synthesis of this compound is economically non-viable, we evaluated various ways of producing it, while determining its natural mechanism of production and ecological relevance. We examined the possibility of: 1) identifying the cells which produce and harbour latrunculin; 2) establishing cell cultures; 3) forming an underwater sponge 'garden'; and 4) takingadvantage of the sponge's own reproduction and larval settlement. Early in the study it became evident that *N. magnifiea* might actually comprise two closely related species of *Negombata*, one of them an undescribed, new species. The work reported here refers to the original *N. magnifica*. 1) The location of Lat B, was studied using specific rabbit anti-Lat B antibodies. Rabbits were immunised with a conjugate of Lat B with Keyhole Limpet Hemocyanin (KLH), and the antibodies were affinity purified over a Lat B-Sepharose column. Thick and thin sections of the sponge were analysed by immuno-histochemical and immuno-gold techniques using light and transmission electron microscopy, respectively. Latrunculin B was prominently labelled in the sponge ectosome -endosome border, especially in the dense cell layer beneath the cortex. Immuno-gold localisation within the sponge revealed that Lat B resides in the sponge cells and not in its prokaryotic symbionts. The labelling density of gold particles in the archeocytes and choanocytes was significantly higher than that of the other sponge cell types (special cells and skeleton associated cells). The antibodies labelled primarily archeocytes and choanocytes, membrane-limited inclusions which are perhaps Lat B secretory and/or storage vesicles. The concentration of Lat B in the sponge periphery correlates with the defensive role of the toxin, since encounters with epibionts, predators and competitive neighbours take place through the surface layer. It may, therefore, be useful to isolate these cells for culture. 2) Primary cell cultures were established from adults and embryos. Mechanical dissociation of inner parts (without the external layer) proved to be superior (less contamination and more cell types) to other techniques. Primary cultures from embryos lasted significantly longer (up to 280 days) and cells survived a freezing phase. Cell lines, however, have not yet been established. 3) Initial steps were taken toward establishing an *in situ* 'garden' of *N. magnifica* from sponge fragments. Although growth rate of sponge fragments was superior to that of natural sponges in their vicinity, fragment survival over a year proved to depend on sponge handling, water depth and environmental conditions (currents, sedimentation etc.). 4) *Negombata magnifica* had a peak in sexual reproduction during the summer. Sexually produced, naturally released, larvae were settled on plates and their growth and development were followed for up to 4 months. ❐ *Porifera, latrunculin, natural product, localization, antibodies, reproduction, immuno-histochemical and immuno-gold techniques, cell culture, Negombata magnifica.*

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